have characterized an intercellular ultrafast Ca^{2+} wave observed in cultured A7r5 cell line and in primary cultured SMCs (pSMCs) from rat mesenteric arteries. This wave, induced by local mechanical or local KCl stimulation, had a velocity around 15 mm/s. Combination of precise alignment of cells with fast Ca^{2+} imaging and intracellular membrane potential recording, allowed us to analyze rapid $[Ca^{2+}]i$ dynamics and membrane potential events along the network of cells. The rate of $[Ca^{2+}]i$ increase along the network decreased with distance from the stimulation site. Gap junctions or voltage-operated Ca^{2+} channels (VOCCs) inhibition suppressed the ultrafast Ca^{2+} wave. Blockage of inositol trisphosphate (IP3) or ryanodine receptors did not affect the Ca^{2+} response. Mechanical stimulation induced a membrane depolarization that propagated and that decayed exponentially with distance. Our results demonstrate an electrotonic spread of membrane depolarization that drives a rapid Ca^{2+} entry from the external medium through VOCCs, modeled as an ultrafast Ca^{2+} wave. This wave may trigger Ca^{2+} release from intracellular stores that drives observed slower Ca^{2+} waves ex vivo and in vivo.

1636-Pos Board B366

Altered CamkII and Ros Microdomains Favor Sparks in Orphaned RyR After Myocardial Infarction

Eef Dries[1], Ilse Lenaerts[1,2], Niall Macquaide[1,2], Demetrio Santiago[1], Piet Claus[3], Karin R Sipido[1].
[1]Cardiovascular Sciences - Experimental Cardiology, KU Leuven, Leuven, Belgium, [2]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom, [3]Cardiovascular Sciences - Cardiovascular Imaging and Dynamics, KU Leuven, Leuven, Belgium.

In ventricular myocytes, ryanodine receptors (RyRs) are typically organized at the Z-line where the sarcoplasmic reticulum forms dyads with T-tubules (TTs). In large mammals, TT density is lower than in rodents and not all RyRs are coupled to the TTs. Recently, we have shown that non-coupled receptors lack a CaMKII and ROS-dependent microdomain modulation and proposed that this may be altered in disease.

Using an established pig model of chronic ischemia and myocardial infarction (MI), we studied myocytes from the area adjacent to the MI and compared these to myocytes from SHAM pigs using whole-cell voltage clamp with Fluo-4 as a $[Ca^{2+}]_i$ indicator and confocal line scan imaging. Spontaneous Ca^{2+} sparks were recorded during a 15s period following stimulation and assigned to different subcellular regions categorized as coupled or non-coupled RyR using a specific algorithm (Dries et al., Circ Res 2013).

In myocytes from SHAM, we confirmed the specific modulation of coupled, but not non-coupled, RyR by CaMKII (using AIP as a specific CaMKII inhibitor) and NOX2-dependent ROS (using gp91 ds-tat peptide as a NOX2 inhibitor) resulting in a higher spark frequency at 2 Hz stimulation. In MI myocytes this modulation of coupled RyR was absent. The fraction of non-coupled RyR was larger (orphaned RyR) and their spark frequency was significantly increased. In MI, only non-coupled RyR were sensitive to CaMKII inhibition (AIP). A significant reduction in spark frequency was observed in these non-coupled RyRs after global ROS scavenging and after mitochondrial ROS inhibition using mitoTEMPO, while NOX2 inhibition had no effect.

In conclusion, after MI there is a novel RyR microdomain organization favoring sparks in orphaned RyR, possibly related to mitochondrial ROS production.

1637-Pos Board B367

Camkii Exacerbates Calcium Waves During Reperfusion of Ischemic Heart

Carlos A. Valverde[1], Alicia Mattiazzi[1], **Ariel L. Escobar**[2].
[1]Centro de Investigaciones Cardiovasculares, La Plata, Argentina, [2]UC Merced, Merced, CA, USA.

Cardiac dysfunction is associated with an abnormal intracellular Ca^{2+} cycling, particularly during the setting of ischemia/reperfusion (I/R). Hearts submitted to ischemia suffer an increase in diastolic Ca^{2+}, accompanied with an increase in sarcoplasmic reticulum (SR) Ca^{2+} load. At the onset of reperfusion there is a massive increase in cytosolic Ca^{2+}, associated with an abrupt release of Ca^{2+} from the SR (Cardiovasc Res, 2006,2010). In a previous work we showed that arrhythmogenic Ca^{2+} waves are the subcellular events associated with this SR-Ca^{2+} release (Circulation, 2013). Moreover, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) has been associated with reperfusion arrhythmias, particularly associated with a CaMKII-dependent phosphorylation of Ser2814 site of ryanodine receptors (RyR2) (JMCC,2011). The aim of the present work was to evaluate the role of CaMKII on the abrupt SR-Ca^{2+} release and enhanced frequency of Ca^{2+} waves during reperfusion. The experiments were done in perfused mouse (C57BL/6) hearts loaded with the Ca^{2+} indicator Fluo-4, on an upright confocal microscope. Hearts were submitted to ischemia and reperfusion (15/30 min) at 32°C, in the presence and absence of a CaMKII inhibitor (KN-93, 2.5 μM). We evaluated Ca^{2+} waves at the epicardial layer of the heart. Inhibition of CaMKII did not prevent the increase in Ca^{2+} wave frequency (0.15 ± 0.05 pre-ischemia vs. 0.29 ± 0.04 waves/100μm/s, ischemia) nor the velocity of the Ca^{2+} wave propagation (122.01 ± 4.62 vs. 126.06 ± 1.02 μm/s). During reperfusion in the presence of KN-93, we observed a reduction in Ca^{2+} waves frequency (0.28 ± 0.06 vs. 0.14 ± 0.02 waves/μm/s, control vs. KN-93), and also in the velocity of its propagation (114.99 ± 3.02 vs. 94.75 ± 1.45 μm/s, control vs. KN-93). The results indicate that the increase in the proarrhythmogenic Ca^{2+} waves at the onset of reperfusion are mainly mediated by a CaMKII-dependent phosphorylation.

1638-Pos Board B368

Stretch-Dependent Regulation of Calcium Signaling in Heart - Who are the Key Players?

Benjamin L. Prosser[1], Christopher W. Ward[2], Jaclyn P. Kerr[1], Guoli Shi[2], W. Jonathan Lederer[3].
[1]Physiology, University of Maryland School of Medicine, Baltimore, MD, USA, [2]University of Maryland School of Nursing, Baltimore, MD, USA, [3]Physiology, BioMET, University of Maryland School of Medicine, Baltimore, MD, USA.

An acute physiologic stretch of a cardiomyocyte triggers an increase in the production of reactive oxygen species (ROS) by the membrane-localized enzyme complex NADPH oxidase 2 (Nox2, X-ROS signaling). The ROS act locally to sensitize nearby Ca^{2+} release channels in the sarcoplasmic reticulum, the ryanodine receptors type 2 (RyR2), resulting in a brief increase in the frequency of calcium sparks. During sustained, cyclical stretch the rate of ROS production remains elevated and is graded by both the degree and frequency of cyclic stretch. The elevated ROS production results in a sustained increase of calcium spark rate, thus coupling the mechanical load on the heart cell to its calcium signaling sensitivity. However, an increase in RyR2 sensitivity alone is insufficient to explain a persistent increase in Ca^{2+} sparks, implicating additional stretch-dependent players in the sustained elevation of calcium signaling sensitivity with increased mechanical stress. We initially investigated three potential players based on their previous links to mechanical stress and modulation by ROS signaling. Here we report on the contribution of nitric oxide (NO), Ca^{2+}/Calmodulin-dependent kinase II (CaMKII), and mechano-sensitive channels (MSC) on stretch-dependent regulation of calcium signaling in ventricular myocytes. We find that both NO and CaMKII have little to no effect on the rapid acute increase in the Ca^{2+} spark rate with stretch ($\leq$10s), but each contribute significantly to maintaining elevated calcium signaling sensitivity with prolonged cyclic stretch ($\geq$1min). We also explore the role of ROS signaling in the stretch-dependent activation of these signaling pathways, and how this affects both diastolic calcium sparks as well as systolic calcium transients and contractility in electrically paced myocytes.

1639-Pos Board B369

The Coupled-Pacemaker Clock System of Sinoatrial Nodal Cells Regulates Both the Action Potential Rate and Rhythm

Yael Yaniv, Alexey E. Lyashkov, Syevda Sirenko, Yosuke Okamoto, Toni-Rose Guiriba, **Edward G. Lakatta**.
NIA/NIH, Baltimore, MD, USA.

Normal automaticity of the sinatrial-node cells (SANC) is regulated by integrated molecular functions within a system of two clock-like oscillators: the sarcoplasmic reticulum, acting as a ''Ca^{2+} clock,'' rhythmically discharges diastolic local-Ca^{2+} releases (LCRs) beneath the cell surface membrane; LCRs activate an inward Na^+/Ca^{2+} exchanger current (INCX) that prompts the ''Membrane clock,'' the ensemble of other sarcolemmal-electrogenic molecules, to generate action potentials (APs). Crosstalk between the two clocks regulates SANC spontaneous AP cycle length (CL). We determine whether clock crosstalk also regulates the rhythm of AP CL in response to perturbation that induce bradycardia via specific inhibition of either membrane or Ca^{2+} clock functions.

We employed ivabradine (IVA, 3, 10 and 30μM) which directly inhibits membrane clock ion channel functions, but has no direct effect on Ca^{2+} clock proteins functions. The IVA-induced increase in the AP CL, however, lowers Ca^{2+} influx, which reduces Ca^{2+}-activated calmodulin-AC-cAMP/PKA signaling, affecting Ca^{2+} and phosphorylation-dependent functions that deive the coupled clock system. Direct and specific inhibition of SERCA2 by cyclopiazonic acid (CPA, 0.5 and 5μM) reduces the magnitude and delays the occurrence of the LCR-Ca^{2+} signal, leading to a reduction in Ca^{2+}-activated calmodulin-AC-cAMP/PKA signaling, and delayed and reduced Ca^{2+} activation of INCX and a prolongation of the AP CL. Importantly, prolongation of LCR period (IVA by 16 ± 2 to 46 ± 5%, CPA by 17 ± 2 to 53 ± 5%), AP CL (IVA by 13 ± 4 to 36 ± 8%, CPA by 16 ± 3 to 44 ± 8%) and the increase in AP CL variability (IVA by 38 ± 10 to 190 ± 50%, CPA by 36 ± 17 to 183 ± 50%) are proportional to each other. The tight inter-relationships among these variables in response to specific perturbation of either the M or the Ca^{2+} clock, therefore, reflect clock crosstalk.